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Review

Effects of social isolation on glucocorticoid regulation in social mammals

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ABSTRACT

This article is part of a Special Issue "Neuroendocrine-Immune Axis in Health and Disease."

The regulation and function of the hypothalamic–pituitary–adrenocortical (HPA) axis and glucocorticoids have been well conserved across vertebrate species. Glucocorticoids influence a wide range of physiological functions that include glucose regulation, metabolism, inflammatory control, as well as cardiovascular, reproductive, and neuronal effects. Some of these are relatively quick-acting non-genomic effects, but most are slower-acting genomic effects. Thus, any stimulus that affects HPA function has the potential to exert wide-ranging short-term and long-term effects on much of vertebrate physiology. Here, we review the effects of social isolation on the functioning of the HPA axis in social species, and on glucocorticoid physiology in social mammals in particular. Evidence indicates that objective and perceived social isolation alter HPA regulation, although the nature and direction of the HPA response differs among species and across age. The inconsistencies in the direction and nature of HPA effects have implications for drawing cross-species conclusions about the effects of social isolation, and are particularly problematic for understanding HPA-related physiological processes in humans. The animal and human data are incommensurate because, for example, animal studies of objective isolation have typically not been modeled on, or for comparability with, the subjective experience of isolation in humans. An animal model of human isolation must be taken more seriously if we want to advance our understanding of the mechanisms for the effects of objective and perceived isolation in humans.

Contents

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Introduction

All vertebrates possess a hypothalamic–pituitary–adrenocortical (HPA) axis that mediates physiological and behavioral change and adaptation through its regulation of corticosteroid production and release (Denver, 2009; Schulkin, 2011). Indeed, the emergence of the vertebrate lineage some 500 million years ago coincided with, and may have been impelled by, the evolution of steroid-specific receptors (e.g., mineralocorticoid, glucocorticoid) from an ancestral nuclear receptor gene already evident in arthropods (Baker et al., 2007; Laudet et al., 1992). The regulation and function of the HPA axis and glucocorticoids (GCs) have been well conserved across vertebrate species (Schulkin, 2011). Glucocorticoids influence a wide range of physiological functions that include glucose regulation, metabolism, inflammatory control, as well as cardiovascular, reproductive, and neuronal effects. Some of these are relatively quick-acting non-genomic effects (Borski, 2000), but most are mediated by slower-acting genomic effects. Up to 20% of the expressed genome in a tissue is susceptible to the direct and indirect influences of GCs, estrogens, and androgens (Chrousos, 2010). Thus, any stimulus that affects HPA function has the potential to exert wide-ranging short-term and long-term effects on much of vertebrate physiology.

The HPA axis is exquisitely sensitive to internal and external environmental perturbations, as would be expected from a system that plays a crucial role in modifying physiology and behavior to subserve energy, immune, and survival needs. In social species, the HPA contributes to physiological and behavioral mechanisms that support a superorganismal (i.e., group) structure that enhances survival of children and grandchildren (Cacioppo and Hawkley, 2009; Cameron et al., 2009; Silk et al., 2009; Waynforth, 2011). Thus, in social species, social isolation is a potent stressor that often leads to increases in cortisol (Cacioppo et al., 2011). In contrast, in solitary species such as the mouse lemur, substantial increases in cortisol are elicited when typically solitary animals are socially housed (Perret and Predine, 1984). A similar difference has been shown in the increased cortical response to isolation in the highly social prairie vole pup versus the solitary montane vole pup (Shapiro and Insel, 1990). The regulation of HPA activity by the presence of some aspects of sociality has been reviewed previously (e.g., Blanchard et al., 2001; DeVries et al., 2003; Hennessy et al., 2009), but to date, research on the regulation of HPA activity in the absence of social others has not undergone a similar review. Here, we review the effects of social isolation on the functioning of the HPA axis in social species, and on glucocorticoid physiology in social mammals in particular. We include the effects on HPA functioning of perceived social isolation in humans. Perceived isolation is not synonymous with objective isolation. People can lead relatively solitary lives and still feel connected to others, and conversely, people can lead ostensibly rich social lives but feel disconnected and lonely nevertheless. Loneliness is defined as a distressing feeling that accompanies the perception that one's social needs are not being met by the quantity or especially the quality of one's social relationships (Peplau and Perlman, 1982). Its effects on HPA functioning are relevant in light of the fact that perceived isolation is associated with increased morbidity and mortality, even after adjusting for objective isolation (Hawkley and Cacioppo, 2010; Luo et al., 2012).

The threat of social isolation

How does social isolation pose a threat to social mammals? Social species are characterized by structured group living in which the survival of individuals is dependent on the safety and security afforded by a social group. Living on the social perimeter is dangerous. Without the ongoing nurturance and protection of parents, offspring do not survive to reproduce. Without the benefit of shared defense and protection afforded by a protective group, individual members are vulnerable to environmental threats and fall prey to faster and stronger predators. Without the social transmission of foraging skills, the challenges of finding food limit survival. Without sharing the effort of finding food, energy demands on the individual are vastly increased. Of course, individuals may cheat the group by seeking and securing the benefits without contributing to the costs. Nevertheless, individuals' survival and reproductive success are greatly enhanced in the group and may be terminally compromised in isolation.

Importantly, individuals may choose to be alone at times and will still feel connected to others. A securely attached infant does just this when he moves away from his mother to explore his surroundings but nevertheless feels as if he can depend on her when needed. Feeling isolated, however, is tantamount to being alone against one's choice or being among others whom one feels cannot be trusted. Accordingly, perceptions of social isolation increase the individual's attention to negative or threatening aspects of the social environment in humans (Cacioppo and Hawkley, 2009), just as objective isolation does in non-human social species. In fact, loneliness, like objective isolation, is unlikely to exist without hypervigilance for social threat. Moreover, perceived isolation and its affective, cognitive, and behavioral sequelae activate the support of physiologically responsive systems (Cacioppo and Hawkley, 2009; Hawkley and Cacioppo, 2010). Although the physiological ramifications may differ between objective and subjective isolation, the highly responsive HPA axis would be expected to register the threat of real or perceived isolation and alter physiology and behavior accordingly. We begin by reviewing the physiology and measurement of HPA functioning.

HPA physiology

The HPA axis controls circulating GC levels through a cascade that starts with hypothalamic secretion of corticotropin-releasing hormone (CRH) from the paraventricular nucleus into the hypophyseal portal circulatory system, which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). Circulating ACTH, in turn, is the key regulator of adrenal secretion of GC hormones (cortisol in humans and most other mammals; corticosterone in rodents). CRH is secreted in a circadian, pulsatile fashion that is largest in the early morning hours. Diurnal variations in ACTH and cortisol follow suit, resulting in a typical diurnal cycle in which cortisol levels reach their peak shortly after awakening, decrease over the course of the day, and reach their nadir about 3 hours after sleep onset.

Glucocorticoid regulation is accomplished systemically via a negative feedback loop involving higher structures of the HPA axis whereby increases in circulating cortisol concentrations inhibit CRH secretion from the hypothalamus and diminish the production of ACTH in the pituitary gland by binding to glucocorticoid and mineralocorticoid receptors (GR and MR, respectively); both processes lead to a decrease in cortisol secretion from the adrenal gland. At the cellular level, cortisol production is controlled by the activity of local enzymes, particularly the 11β-hydroxysteroid dehydrogenases 1 and 2 (11β-HSD1 and 11β-HSD2). Whereas 11β-HSD1 converts the biologically inactive cortisone to active cortisol, 11β-HSD2 converts cortisol to inactive cortisone (Seckl and Walker, 2001).

Systemic GC regulation is influenced by the degree of HPA axis sensitivity to glucocorticoid feedback. Glucocorticoid sensitivity at the level of the pituitary can be assessed by administering dexamethasone (i.e., the dexamethasone suppression test, DST), a potent synthetic glucocorticoid that inhibits cortisol through suppression of pituitary ACTH secretion. The degree of cortisol inhibition is in large part a function of the responsiveness of glucocorticoid receptors in the pituitary, although neurotransmitters and cytokines that affect HPA target tissues (Turnbull and Rivier, 1995) also contribute to the cortisol response to dexamethasone (Yehuda et al., 2003). Using a DST, glucocorticoid resistance is indicated by a reduction in the ability of dexamethasone to inhibit endogenous cortisol release.

Glucocorticoid sensitivity can also be assessed in individual tissues. Leukocytes, for example, possess glucocorticoid receptors that regulate a variety of functions, including proliferation, trafficking, and cytokine production. In vitro application of dexamethasone can be used to assess the sensitivity of leukocytes to the influence of GCs on these functions. Some research has indicated that GC sensitivity differs among target tissues (Ebrecht et al., 2000), but there is evidence that glucocorticoid sensitivity of the leukocyte is correlated with sensitivity at the pituitary (Lowy et al., 1988; Yehuda et al., 2003). In addition, leukocyte sensitivity to cortisol differs as a function of levels of cortisol. For instance, Sauer et al. (1995) found a positive correlation between in vitro lymphocyte proliferation to the mitogen, PHA (phytohemagglutinin), and degree of cortisol-induced inhibition of DNA synthesis in students under academic stress when cortisol levels were high. In contrast, this correlation was negative in the same students when not under academic stress and cortisol levels were lower. In other words, sensitivity of lymphocytes to the inhibitory effects of cortisol decreases with increased HPA activity. Effects consistent with this pattern of results were recently reported by Cohen et al. (2012), who found the expected positive correlation between cortisol levels and the circulating proportion of neutrophils relative to lymphocytes (a measure of glucocorticoid sensitivity) among non-stressed individuals, but found no correlation among those who had experienced a major stress (Cohen et al., 2012).

Effects of social isolation on HPA physiology

Non-human social mammals

The direct effects of social isolation on basal activity of the HPA axis have been examined in a number of species (Table 1). In non-primate mammals, social isolation is associated with higher basal levels of plasma ACTH and/or corticosterone in some studies (Chida et al., 2005; Dronjak et al., 2004; Ferland and Schrader, 2011; Kanitz et al., 2004; Perelló et al., 2006; Pournajafi-Nazarloo et al., 2011; Ruscio et al., 2007; Weintraub et al., 2010), lower levels in other studies (Djordjevic et al., 2010; Hermes et al., 2005; Sánchez et al., 1998; Serra et al., 2005; Weintraub et al., 2010), and has been indistinguishable from levels in group-housed animals in yet other studies (Castro and Matt, 1997; Grippo et al., 2007a; 2007b; Pournajafi-Nazarloo et al., 2011; Scaccianoce et al., 2006; Stowe et al., 2005; Toth et al., 2011; Weiss et al., 2004). Inconsistent findings have been noted previously (Mason, 2000; Serra et al., 2007), and may be attributable to species differences and/or differences in the nature of the social isolation procedures, the age at which the animals were isolated, and the duration of their isolation. For instance, in socially monogamous female and male prairie voles, the effects of social isolation on corticosterone depend on chronicity of isolation. A single 1-h period of isolation or 4 weeks of daily 1-h periods of isolation resulted in significant increases in plasma corticosterone levels, but chronic isolation for 4 weeks did not affect plasma corticosterone levels (Pournajafi-Nazarloo et al., 2011).

Serra et al. (2007) suggested that chronic isolation, even if it has no discernible effect on basal glucocorticoid levels, induces a "facilitatory trace" (Akana et al., 1992) that enhances HPA responsiveness to new stressors. Research has borne this out using acute stressors that range from immobilization, cold stress, acoustic startle, and foot shock, to induced cardiac arrest or stroke (e.g., Norman et al., 2010). Consistent with the idea of priming the HPA axis with chronic isolation, corticosterone responses to acute stress have been shown to be significantly larger in mice, rats, and hamsters subjected to long-term isolation (21 days to 18 weeks) compared to group-housed control animals (Detillion et al., 2004; Dronjak et al., 2004; Ferland and Schrader, 2011; Grippo et al., 2007a; 2007b; Hermes et al., 2005; Toth et al., 2011; Weintraub et al., 2010; Weiss et al., 2004; Williams et al., 2009; but see decreased corticosterone responses in Djordjevic et al., 2010; Sánchez et al., 1998). Social isolation has also been shown to increase cortisol responses to stress in larger domestic mammals such as pigs and cattle (Creel and Albright, 1988; Kanitz et al., 2009).

In non-human primate species, both acute and chronic isolation have been shown to increase levels of basal cortisol. Captive adult marmosets (*Callithrix jacchus*) responded to a brief 15-min period of social isolation with significant increases in salivary cortisol (Cross et al., 2004). Similarly, marmosets (*Callithrix kuhli*) subjected to 11 h of isolation exhibited increased cortisol (Smith and French, 1997). In Geoffroy's marmosets (*Callithrix geoffroyi*), prolonged social isolation (6–20 weeks) in adulthood prior to cohabitation with an opposite sex partner increased cortisol levels and social proximity, and tended to increase allo-grooming behavior. Urinary cortisol levels remained consistently higher in previously isolated animals over the course of the 90-day cohabitation period than in the animals who had remained with their natal group prior to cohabitation (Smith et al., 2011). Juvenile rhesus monkeys subjected to four sequential 4-day separations from their mothers or peer groups exhibited significantly and persistently elevated levels of plasma cortisol (Higley et al., 1992). In contrast, squirrel monkeys (*Saimiri sciureus*) who had been subjected to repeated 4–6 h bouts of isolation over a period of 2 months in early life (pre-weaning) showed significantly lower levels of cortisol at 2 and 3 years of age than their non-isolated counterparts (Levine and Mody, 2003). Similarly, rhesus monkeys isolated for 1 month at birth exhibited lower levels of cortisol (in hair) at puberty (2 and 3.5 years of age) than their non-isolated counterparts (Feng et al., 2011). The developmental timing of isolation is critical, however, and pre-weaning effects may differ systematically from post-weaning effects (e.g., Weiss et al., 2001), and effects measured years after the social isolation procedure may elicit a different pattern of effects than those measured immediately after isolation. In addition, non-human primate species differences in the nature of their social bonds may contribute to differences in the effects of social isolation as exemplified in, for instance, infants with attachment versus affiliative bonds (Hennessy, 1997) and adult animals with monogamous versus polygynous mating systems (Mendoza and Mason, 1986; Mendoza et al., 2000).

HPA regulation

Effective glucocorticoid feedback inhibition is critical to HPA regulation, and social isolation has been shown to influence these processes, as well. In rats, a 3-week period of isolation in early life followed by 2 weeks of group-rearing resulted in exaggerated recovery of corticosterone levels to below basal levels two hours after an acute restraint stress, indicating heightened negative feedback of the HPA axis in response to acute stress (Lukkes et al., 2009). In contrast, rats chronically isolated for 4 weeks in early life and not subjected to an acute stress exhibited impaired negative feedback regulation of HPA activity; a higher dose of dexamethasone (a synthetic glucocorticoid) was needed to suppress corticosterone in isolated than in group-housed rats (Serra et al., 2005). Moreover, intracerebroventricular infusions of corticotrophin releasing factor increased plasma corticosterone levels to a greater degree in isolated than group-housed rats. Despite evidence of impaired negative feedback, isolated rats had lower basal levels of plasma ACTH, suggesting that ACTH regulation in these animals is more complicated and may involve other regulatory pathways (Serra et al., 2005).

Juvenile squirrel monkeys (*Saimiri sciureus*) individually housed for 7 days exhibited morning levels of cortisol that were 32% higher than levels when the same animals were housed in groups (Lyons et al., 1995; see also, Lyons et al., 1999). ACTH levels, on the other hand, were significantly lower when animals were individually housed than when group housed. Dexamethasone was administered to suppress secretion of endogenous ACTH, and was followed the next morning with a bolus injection of ACTH to assess adrenal responsiveness. Results revealed that individually housed monkeys

Table 1
Effects of social isolation on HPA functioning in diverse social mammals.

Reference	Species	Age at isolation	Isolation duration	Age at testing	Basal GC & ACTH	GC reactivity to stress
Chida et al. (2005)	Mice, male	Post-weaning (5 weeks)	9 weeks	Adult	n.s. GC different at baseline. Greater GC increase from baseline in isolated than group-housed animals at 9 weeks; group-housed animals continued to show GC increase; at 19 weeks, control GC > isolated GC	
Williams et al., 2009	Mice	Post-weaning	Chronic	Adult		Increased GC rx to stress
Pournajafi-Nazarloo et al. (2011)	Voles	Adult	1 h, or 4 weeks of daily 1-h (separated from same-sex sibling partner)	Adult	Increased GC	
Pournajafi-Nazarloo et al., 2011	Voles	Adult	4 continuous wks	Adult	n.s. difference	
Ruscio et al. (2007)	Voles	Post-weaning	4 or 21 days	Juvenile	Increased GC (& increased CRF in hypothalamus)	
Stowe et al. (2005)	Voles	adult, 3–4 months of age	24 h or 2 weeks	Adult, 3–4 months of age	n.s. difference	
Grippo et al. (2007a)	Voles (females)	Adult	60 days	Adult	n.s. difference	Increased GC rx to resident intruder
Grippo et al. (2007b)	Voles (males & females)	Adult	4 weeks	Adult	n.s. difference	Increased GC rx to resident intruder
Djordjevic et al. (2010)	Rats	Adult	21 days individual housing, followed, or not, by 30' immobilization	Adult	Decreased GC	n.s. different in cortisol rx to immobilization
Dronjak et al. (2004)	Rats	Adult	long-term	Adult	Increased GC relative to long-term crowded animals	Increased GC rx to acute immobilization and cold stressors relative to long-term crowding
Ferland and Schrader (2011)	Rats	Adult (56 days)	Overnight for 14 consecutive nights, with or without chronic variable stress (warm/cold swim, cold room, rotation)	Adult		Increased GC in response to separation with or without chronic variable stress
Hermes et al. (2005)	Rats	Post-weaning (~28 days)	3 months	Adult, 4 months	Decreased GC	Increased GC rx to restraint stress in females
Lukkes et al. (2009)	Rats, male	Juvenile, day 21	3 weeks, followed by 2 weeks group housing	Adult (8 weeks)		n.s. difference in cortisol rx to restraint stress
Perelló et al. (2006)	Rats	Post-puberty (35 days)	4 weeks	Adult, 9 weeks	GC increased in plasma, decreased in adrenal gland	
Sánchez et al., 1998	Rats	Post-weaning (early @ 16 days)	2 months	Adult	Decreased GC	Decreased GC rx to immobilization stress
Scaccianoce et al. (2006)	Rats, male	Adult (2 months)	8 weeks		GC n.s. different from pair-housed rats	
Serra et al. (2005)	Rats	Early life	chronic, 4 weeks	Adult	Decreased basal ACTH	
Toth et al. (2011)	Rats	Post-weaning (21 days)	7–8 weeks	Adult	GC n.s. different than socially housed animals	Increase rx to aggressive encounter
Weintraub et al. (2010)	Rats	Adolescent (30 days)	3 weeks (days 30–50); then housed in same-sex groups	Adolescent (50 days) & adult (70 days)		Adolescence: greater GC recovery in males and less GC recovery in females following acute restraint stress. Adult: decreased GC rx in males and increased GC rx in females following restraint stress.
Weiss et al. (2004)	Rats	Post-weaning	Chronic	Adult (4 months)	ACTH increased in males, but not females	Increased GC & ACTH rx to (open field & elevated plus maze) stress in males, but not females
Castro and Matt, 1997	Hamsters, Siberian dwarf (male)	Adult	Males separated from mate for 4 weeks	Adult	Increased GC in isolated relative to mate-housed animals?	
Detillion et al. (2004)	Hamsters, Siberian dwarf (female)	Post-weaning (2–3 months)		Adult	GC n.s. different than pair-housed animals	Increased rx to immobilization

(continued on next page)

Table 1 (*continued*)

Reference	Species	Age at isolation	Isolation duration	Age at testing	Basal GC & ACTH	GC reactivity to stress
Creel and Albright, 1988	Cattle	Neonatal	10 weeks	Post-puberty, 1 year of age	n.s. GC increase relative to control calves at 10 weeks of age	Increased rx to acute stress (approach & flight distances from humans) at 10 weeks of age
(Kanitz et al., 2009; Tuchscherer et al., 2010)	Pigs	Pre-weaning	Single 4-h bout	Infant: 7, 21, and 35 days of age		Increased GC rx to immobilization
Kanitz et al.(2004)	Pigs	Pre-weaning	2-h daily, Days 3–11	Pre-weaning	Increased GC	
Tuchscherer et al. (2004)	Pigs	Pre-weaning	2-h daily, Days 3–11	Pre-weaning	GC & ACTH increased at Day 12 & Day 56 vs. control piglets n.s. different cortisol response to peripheral LPS in isolated vs. control animals	
Cross et al. (2004)	Marmosets	Adult	15 min	Adult	Increased GC	
Smith et al. (2011)	Marmosets (Geoffroy's)	Adult	6 to 20 weeks	Adult	Increased GC	
Smith and French (1997)	Marmosets	Adult	11 h; 11 h plus 5 min handling stress after net capture	Adult	Increased GC	
Feng et al. (2011)	Monkeys (rhesus)	Pre-weaning (at birth)	1 month, then peer or mother-reared	Puberty: 2 & 3.5 years	Basal hair cortisol lower @ both time points in peerthan mother-reared animals	Delayed plasma cortisol recovery from capture and restraint stress in peerreared relative to mother-reared animals.
Higley et al. (1992)	Monkeys (rhesus)	Juvenile: 6 & 18 months	4 sequential 4-day separation from mothers or peer group	Juvenile: 6 & 18 months (prior to and on last day of separation)	See GC reactivity to isolation stress	Increased plasma cortisol response to social separation; persistently elevated cortisol throughout separation
Levine and Mody, 2003	Monkeys (squirrel)	Pre-weaning	4–6 h bouts for 2 months	Adult (2–3 years of age)	Decreased GC	
Lyons et al. (1995)	Monkeys (squirrel)	Juvenile	7 days individual housing	Juvenile	GC increased; ACTH decreased	
Lyons et al. (1999)	Monkeys (squirrel)	Adolescent, late (31–33 months)	6 days housed alone (vs. in pairs and in 4-monkey groups)	Adolescent, late	GC increased 1 day after separation vs. paired condition; ACTH increased up to 1 h post-separation, then decreased 1 day postseparation vs. paired condition (cortisol inversely related to ACTH)	
Mendoza and Mason (1986)	Monkeys (squirrel)	Adult	1-h in home cage separated from pairmate	Adult	See GC reactivity to isolation stress	No effect on plasma corticosteroid 1 h postseparation
Mendoza and Mason (1986)	Monkeys (titi)	Adult	1-h in home cage separated from pairmate	Adult	See GC reactivity to isolation stress	Increased plasma cortisol 1 h post-separation
Sapolsky et al. (1997)	Baboons	Adult & juvenile	Social isolation relative to median values	Adult & juvenile	Increased GC	
Arnetz et al. (1983)	Humans	Adult, older	Senior citizen housing (social activity program vs. control)	Adult, older	Increased GC	
Ebrecht et al. (2004)		Adult	Loneliness	Adult	n.s. association with morning rise in cortisol	
Grant et al. (2009)	Humans	Adult, middle-aged	Smaller social network	Adult, middle-aged	Increased GC (daily integrated "area-under-the curve")	
Kiecolt-Glaser et al. (1984a,1984b)	Humans	Adult	Loneliness in psychiatric patients	Adult	Increased GC (urinary)	
Pressman et al. (2005)	Humans	Adult, young	Loneliness	Adult, young	increased GC	
Adam et al. (2006)	Humans	Adult, middle-aged	Loneliness adult, middle-aged	Increased morning rise in cortisol as a function of trait loneliness and as a function of daily fluctuations in loneliness		
Steptoe et al. (2004)	Humans	Adult, middle-aged	Loneliness	Adult, middle-aged	Increased morning rise in cortisol as a function of trait loneliness; n.s. association with total cortisol or cortisol slope over working day	n.s. association with cortisol response to Stroop mirrortracing tasks
Doane and Adam (2010)	Humans	Adult, middle-aged	Loneliness	Adult, young	Increased morning rise in cortisol as a function of daily fluctuations in loneliness, and flatter diurnal slope as a function of trait loneliness	

Legend: GC = glucocorticoid; n.s. = not/non significant(ly); rx = reactivity.

exhibited a significantly larger and longer-lasting increase in cortisol than monkeys consistently housed in groups. Together, the results indicate that increased morning cortisol in isolated monkeys is due to enhanced adrenal responsiveness to ACTH, and low basal ACTH levels suggest effective glucocorticoid feedback inhibition of pituitary ACTH (Lyons et al., 1995). Similarly, in pre-weaning squirrel monkeys, periodic removal of animals from the natal group enhanced cortisol-induced suppression of ACTH in response to CRF injection (Lyons et al., 2000). However, in adult and juvenile baboons (*Papio cynocephalus*), relative social isolation (i.e., negative deviations from median values on a composite measure of social connectedness) was associated with elevated levels of basal cortisol and tended to be associated with dexamethasone resistance (Sapolsky et al., 1997). Glucocorticoid resistance differences between squirrel monkeys and baboons may be attributable to differences in the age of the animals (juvenile monkeys vs. adult and juvenile baboons), but may also reflect species differences. Unique features of HPA physiology in the squirrel monkey relative to the baboon and other Old World primates include exceedingly high but non-pathological levels of cortisol and low affinity GRs for cortisol (Reynolds et al., 1999).

Humans

In humans, researchers typically operationalize objective social isolation in terms of extent; social network size is a common index of degree of isolation. In healthy middle-aged men and women from the Whitehall II cohort, social isolation was assessed by summing the number of close persons in participants' social networks. Socially isolated individuals—those who had fewer close persons in their network—exhibited a larger cortisol awakening response and greater total cortisol output over the course of a day (Grant et al., 2009).

A study conducted several decades ago indirectly tested the effect of relative social isolation on HPA activity by assigning socially inactive elderly adults in a senior citizen apartment to a social activation program (social events, special interest groups) and comparing the neuroendocrine effects with those observed in a control group of socially inactive residents on another floor of the building. In comparison with the residents in the social activation program, residents in the control group showed a significant decrease in height over a 6-month period as well as a decrease in plasma dehydroepiandrosterone (DHEA) and an increase in plasma cortisol at the 3-month follow-up (Arnetz et al., 1983). The height finding is particularly interesting in light of the adverse effect of cortisol on bone mineral density and bone loss with age (Dennison et al., 1999).

Studies of subjective isolation provide another window on the effects of isolation on HPA function. In young adults, loneliness has been associated with increased levels of early morning and late night cortisol (Pressman et al., 2005) and, if chronic, with elevated daily cortisol output (Cacioppo et al., 2000). Trait loneliness has also been associated with a flattened diurnal cortisol rhythm, whereas daily variations in feelings of loneliness have been associated with the cortisol awakening response; the greater the feeling of loneliness on the prior day, the larger the increase in salivary cortisol during the 30 min post-awakening the following morning (Doane and Adam, 2010). A larger cortisol awakening response has also been observed in middle-aged and older adults, prospectively as a function of prior day's feelings of loneliness (Adam et al., 2006) and in association with trait levels of loneliness (Steptoe et al., 2004).

HPA regulation

To date, only one study of loneliness differences in sensitivity to glucocorticoid feedback has been reported. This recent research has employed a novel functional measure of glucocorticoid insensitivity that is based on the logic that exposure to glucocorticoids alters the distribution of leukocytes in circulation. Namely, cortisol stimulates an increase in circulating concentrations of neutrophils and a decrease in concentrations of lymphocytes and monocytes such that the greater the exposure to cortisol the greater should be the ratio of neutrophils to lymphocytes (NLR) or monocytes (NMR). In a study of approximately 1,500 older Taiwanese adults, loneliness was associated with an attenuation of the relationship between overnight urinary cortisol levels (an integrative measure of HPA activity during the hours prior to blood collection) and the NLR and NMR, indicating resistance of the leukocytes to cortisol (Cole, 2008).

Physiological and behavioral effects of HPA activation in isolated animals

Non-human social mammals

The exaggerated effect of acute stress on HPA activity in socially isolated animals has implications for processes regulated by glucocorticoids. For instance, corticosterone responses to acute stress in socially isolated animals affect neuronal activity. Whereas running induced hippocampal neurogenesis in group housed rats, it suppressed hippocampal neurogenesis in individually housed rats, an effect that was attributable to larger corticosterone responses to the additional stress of handling and injection in isolated relative to group-housed runners (Stranahan et al., 2006).

In addition, increased cortisol in isolated animals influences immune processes. In vitro assays of peripheral blood mononuclear cells stimulated with concanavalin A (ConA) or lipopolysaccharide (LPS) revealed a dose-dependent cortisol resistance in pigs at 7, 21, and 35 days of age after a single 4-h period of postnatal social isolation relative to control animals (Tuchscherer et al., 2010). On the other hand, the cortisol response to peripheral LPS after repeated social isolation (i.e., 2 h daily from days 3–11) did not differ from that seen in control piglets (Tuchscherer et al., 2004). Immobilization stress resulted in increased cortisol and impaired wound healing in individually housed but not pair-housed hamsters. In isolated hamsters, removal of cortisol via adrenalectomy abolished the effect of stress on wound healing, and blocking of the cortisol stress response via treatment with oxytocin facilitated wound healing. Conversely, treating the pair-housed hamsters with an oxytocin antagonist delayed wound healing (Detillion et al., 2004). The wound healing effects in this study are consistent with the known immunosuppressive effects of cortisol and also indicate that species-typical sociality buffers HPA activity through the actions of the prototypical social bonding hormone, oxytocin. Thus, social isolation produces exaggerated HPA responses while simultaneously diminishing the potency of HPA-buffering effects of sociality. It is notable in this context that, in humans, prolonged isolation from parents during childhood is associated with diminished central sensitivity to the dampening effects of oxytocin on cortisol secretion in adult men (Meinlschmidt and Heim, 2007).

Humans

Loneliness has been associated with impaired cellular and humoral immunity. In lonely relative to nonlonely nonpsychotic psychiatric patients, elevations in urinary levels of cortisol were accompanied by lower natural killer cell activity and poorer T-lymphocyte response to mitogen stimulation (Kiecolt-Glaser et al., 1984a, 1984b), consistent with the known immunosuppressive effects of cortisol. In response to acute stress, however, loneliness has been associated with a larger natural killer cell response (Steptoe et al., 2004). Other studies have shown that reactivation of latent herpes virus (Glaser et al., 1985) and Epstein-Barr virus (Kiecolt-Glaser et al., 1984c) is greater in lonely than nonlonely individuals, effects that are consistent with glucocorticoid immunosuppression of cellular control of latent virus. Humoral responses to an influenza vaccine also differ as a function of loneliness: greater loneliness was associated with a smaller antibody response to

Fluzone vaccine (Pressman et al., 2005). Although cortisol levels were also elevated in lonely individuals, cortisol was not found to mediate the effect of loneliness on antibody response.

Social isolation and expression of HPA-related genes

Non-human social mammals

The primary upstream determinant of circulating levels of glucocorticoids involves the regulation of the expression of genes that control the synthesis of hypothalamic CRH, pituitary ACTH, adrenal corticosterone (cortisol in humans), and the corticoid receptors. These genes are within reach of the influence of social isolation. In the study of prairie voles mentioned above (Pournajafi-Nazarloo et al., 2011), a single 1-h period of isolation or 4 weeks of daily 1-h periods of isolation resulted in increased CRH mRNA expression in the hypothalamus and increased CRH-R1 (corticotrophin releasing hormone-receptor 1) mRNA expression in the pituitary. Chronic isolation for 4 weeks, on the other hand, had no effect on hypothalamic or pituitary CRH or CRH-R1 mRNA (Pournajafi-Nazarloo et al., 2011; but see Pan et al., 2009). These results are consistent with elevated corticosterone levels observed in acutely isolated animals and unchanged corticosterone levels in chronically isolated animals relative to pair-housed animals. Similarly, in rats, neither corticosterone levels nor hippocampal MR (mineralocorticoid receptor) and GR mRNA expression differed between chronically isolated and control animals (Weiss et al., 2004).

In domestic piglets, social isolation-induced changes in gene expression were consistent with accomplishing effective negative feedback at the level of the hippocampus and hypothalamus, primary central feedback target regions. Specifically, a single 4-h period of social isolation on day 7 or day 21 of life was sufficient to upregulate glucocorticoid receptor (GR) in hypothalamus, and to upregulate 11β-HSD1 mRNA expression in hypothalamus and hippocampus compared to expression changes in control animals (Kanitz et al., 2009). In contrast, in piglets briefly isolated for 15 min just prior to euthanasia compared with expression in non-isolated animals, frontal cortex showed decreased expression, and hippocampus showed no change in expression, of genes for GR and MR and the dehydrogenases, 11β-HSD1 and 11β-HSD2 (Poletto et al., 2006). Study differences in the effects of isolation may be attributable in part to the duration of isolation and in part to the areas of the brain examined for gene expression changes. In non-human primates, a study of marmosets isolated for 30–120 min at 1 month of age and tested at adolescence showed modestly reduced hippocampal expression of mRNA for mineralocorticoid and glucocorticoid receptors, but no change in these RNA's in hypothalamus or prefrontal cortex (Arabadzisz et al., 2010).

Humans

As was noted above, the function of GCs has been well conserved in social mammals. For instance, in non-human animals, prenatal exposure to glucocorticoids reduces birthweight and causes permanent hypertension, hyperglycemia, and increased HPA activity; in humans, low birthweight infants have higher levels of glucocorticoids and this increase in HPA activity precedes the development of high blood pressure, insulin resistance, glucose intolerance, and hyperlipidemia (Seckl and Meaney, 2004). These health conditions all have a substantial inflammatory component. This commonality lends import to recent findings linking perceived isolation with a pro-inflammatory gene expression profile (Cole et al., 2007, 2011). Specifically, genome-wide microarray analyses revealed significant upregulation of pro-inflammatory markers (e.g., pro-inflammatory cytokines, inflammatory mediators, and bioinformatic indication of activated NF-κB transcription factor) and downregulation of anti-inflammatory markers (e.g., bioinformatic indications of reduced transcriptional activity of the glucocorticoid receptor) in lonely relative to socially connected middle and older-age adults (Cole et al., 2007, 2011).

There are two major pathways through which we hypothesize loneliness might affect NF-κB and inflammatory signaling more broadly. One involves direct effects of loneliness-related increases in sympathetic nervous system/beta-adrenergic signaling in increasing the basal activation level of NF-κB (Bierhaus et al., 2003) and other pro-inflammatory transcription factors (e.g., Cole et al., 2010), effects that are independent of any change in GR function. The second pathway involves loneliness-related reductions in basal activity of the anti-inflammatory GR transcription factor, and the resulting permissive de-repression of basal NF-κB and other pro-inflammatory transcription factors to establish a new basal homeostatic set-point involving increased expression of pro-inflammatory genes (as reviewed in De Bosscher et al., 2006). In this scenario, loneliness-related changes in physiologic function are hypothesized to lead to reduced functional activity of the GR, and a resulting increase in basal inflammatory set-point of the NF-κB transcription factor that it inhibits. These processes signify influences on HPA functioning that may explain increased risk of inflammatory disease in chronically lonely individuals.

Discussion and implications for future research

The responsivity of the HPA axis to the perturbation of social isolation is apparent across a range of social mammals. Social isolation represents a survival threat to social species, and physiology has been sculpted to mount an appropriate response. Increased levels of glucocorticoids mobilize energy and dampen inflammation in a presumably adaptive fashion. These effects are particularly evident in response to acute periods of social isolation and to additional acute stress in animals already dealing with the stress of isolation. In humans, perceived isolation is a painful stimulus that has been posited to be adaptive (Cacioppo and Hawkley, 2009) because it motivates the formation and nurture of social connections, connections that help regulate physiology and ensure survival. However, when the need for social connections is not satisfied and the pain of isolation persists, HPA dysregulation is more likely to ensue. In human and non-human animals, chronic isolation alters HPA functioning and regulation in ways that are less easily interpreted as adaptive. Instead, chronic isolation has been associated with changes in gene expression and an increased likelihood of glucocorticoid resistance, outcomes that heighten risk for inflammatory processes, reduced immune responses, and disease.

Reduced immune responses and impaired wound healing may seem maladaptive given that an isolated animal is more vulnerable to attack from conspecifics or predators. Evolution should arguably have favored isolation-induced immune enhancement. Such an argument was forwarded by Cole et al. (2011), where perceived social isolation, a circumstance that would be expected to increase risk of bacterial infection through its association with increased hostility and social conflict, was associated with a gene transcription profile that favored innate antibacterial and T-helper 2 adaptive immune responses over antiviral and T-helper 1 immune responses. In contrast, for socially connected individuals in socially affine conditions, an environment that would be expected to increase risk of viral infection, the transcriptional profile favored innate antiviral over antibacterial immune responses.

The principle of shifting the immune response toward anticipated bacterial threats may hold, but additional factors superimposed on social isolation may take precedence and determine the observed immune response. Specifically, data indicate that impairment in the early inflammatory response, during which infection is controlled, is attributable to acute or chronic stress, not social isolation per se, in both human and animal models of wound healing (DeVries et al., 2003; Vileikyte, 2007). For instance, isolation alone was insufficient to alter wound healing rates in hamsters (Detillion et al., 2004). Only isolated animals subjected to restraint stress exhibited impaired wound healing relative to isolated-no stress and paired-stress or paired

no-stress animals. In humans, a meta-analytic review of 22 studies concluded that stress (operationalized as stress appraisals, life events, chronic stress, anxiety, depressive symptoms) was significantly associated with impaired wound healing (Walburn et al., 2009). Notably, the two studies that included a measure of perceived social isolation or loneliness failed to find an association with wound healing and found, instead, that perceived stress (Ebrecht et al., 2004) or dysphoria (Bosch et al., 2007) were associated with impaired wound healing in these same subjects.

Cortisol appears responsible for dampening the inflammatory response that characterizes early stages of wound healing (Padgett et al., 1998). In the hamster study by Detillion et al. (2004), isolated animals exhibited a significantly higher cortisol response to restraint stress than pair-housed animals. This should not be surprising given that restraint stress (i.e., physical immobilization) is a very significant condition for any animal, much less an already vulnerable socially isolated animal. Thus, even if social isolation inclines the immune system toward an antibacterial response, the addition of a stress like immobilization could arguably be expected to divert the vulnerable isolated animal's energy (including glucocorticoids) toward heightened vigilance, trying to find the group, etc., rather than to wound healing. Additional stress may be the proverbial straw that breaks the socially isolated animal's immune system, at least in early stages of the inflammatory response.

Isolation-induced HPA dysregulation may manifest differently at different ages, a phenomenon that can perhaps be best understood in evolutionary terms. Social isolation may represent a different survival challenge to an infant or pre-weaning animal than it does to a sexually mature or aged animal. To a human infant or pre-weaning animal, survival requires not only a reliable caregiver but also protection against the adverse consequences of elevated glucocorticoids on the developing central nervous system (Sapolsky and Meaney, 1986). This may explain the hyporesponsiveness of pre-weaning animals and human infants to stress (Gunnar and Donzella, 2002; Sapolsky and Meaney, 1986), a phenomenon that has been postulated to buffer the HPA axis and ensure its healthy development. Good mothering, defined in rats as frequent and regular licking and grooming, effectively protects the infant brain by maintaining low glucocorticoid levels in the infant (Meaney, 2001), illustrating the importance of the quality of the parental connection for the survival and neural protection of offspring.

Post-weaning and sexually mature animals, on the other hand, are confronted with survival challenges that emphasize heightened HPA sensitivity to environmental input as this may determine their reproductive strategies (Ruscio et al., 2007). Physiological trade-offs between the reproductive and immune systems are common (Martin et al., 2008), and sexual maturity would be expected to differentially influence the primacy of immunity versus reproduction. In broad evolutionary terms, the threat to survival posed by social isolation should, in a post-weaning sexually immature animal, preferentially shift energy to immune defenses to preserve its own survival to reproductive age. In contrast, evolutionary pressures would be expected to have primed an isolated sexually mature animal to preferentially shift energy to accelerated and intensified reproductive efforts before it's too late to perpetuate one's genes. These scenarios are supported by our argument that the pain of social isolation motivates individuals to return to their social group where they will find safety and enhanced likelihood of survival, and where reproductive activity is most likely to occur. Importantly, increased glucocorticoid output that accompanies social isolation does not necessarily inhibit reproduction. Recent research has shown that although stress-related cytokines such as TNF-alpha activate the HPA and inhibit the HPG (hypothalamic–pituitary–gonadal) axis, glucocorticoids protect activity of the HPG. Specifically, GCs inhibit COX-2 (cyclooxygenase-2) in the brain, thus inhibiting PG synthesis and protecting the secretion of luteinizing hormone, a key reproductive hormone released by the pituitary (Matsuwaki et al., 2006). In the context of social isolation, increased glucocorticoid output provides the energy to accomplish immune and reproductive goals.

Cortisol is in itself incapable of adverse or advantageous effects; only binding to the receptor enables its functional consequences. This places a premium on understanding regulation of the glucocorticoid receptor, and isolation-induced changes in gene expression in humans and non-human primates appear to be a promising avenue of future research. Additional research is needed to determine the extent to which social isolation acts directly on upstream HPA-related gene expression and indirectly through, for example, proinflammatory cytokines that influence glucocorticoid gene expression directly or indirectly through their activation of the NF-κB transcriptional pathway. Functional assessments to evaluate the extent to which changes in gene expression are reflected in protein production (e.g., GR's) will help elucidate a possible mechanism for enhanced morbidity and mortality risk in isolated individuals. In this regard, GR subtypes could be quantified to evaluate the effect on glucocorticoid resistance of imbalances in the ratio of GRα to GRβ; GRα is the active form responsible for initiating glucocorticoid effects, whereas inactive GRβ competes with GRα for the binding of glucocorticoid response element, thus inhibiting GR actions (Barnes and Adcock, 2009).

Our review of the research linking social isolation and HPA functioning revealed some consistency within species and across some animal species. As is shown in Table 1, however, inconsistencies are also evident in the direction and nature of HPA effects within and across species. These inconsistencies have implications for drawing cross-species conclusions about the effects of social isolation, and are particularly problematic for understanding HPA-related physiological processes in humans. The animal and human data are incommensurate because, for example, animal studies of objective isolation have typically not been modeled on, or for comparability with, the subjective experience of isolation in humans. An animal model of human isolation must be taken more seriously if we want to advance our understanding of the mechanisms for the effects of objective and perceived isolation in humans. A model of isolation in animals that is conceptually equivalent to perceived isolation in humans is not sufficient, of course. Measurements (type, timing) have to be expanded to see whether and how the animal model is relevant for humans. For instance, research in humans has tended to find an association between loneliness and an increased cortisol response to awakening and/or a flatter diurnal slope, but research in animals has focused on basal glucocorticoid levels or stress responses. Do isolated animals exhibit an elevated cortisol awakening response? Does the cortisol awakening response represent anticipated demands of the environment as has been suggested by human research (Adam et al., 2006)? Cortisol measurement in humans needs to be considered more carefully as well. Publications are littered with inconsistencies at least in part because researchers employ different cortisol parameters (morning levels, awakening response, evening levels, diurnal slope, area under the curve, plasma vs. saliva, etc.) or choose to report only those parameters that have significant associations with the predictor or outcome of interest. Careful selection and comprehensive reporting of cortisol parameters are important considerations to facilitate cross-species and cross-study comparisons.

Of course, to capitalize on an animal model, research in humans must be informed by animal findings and reciprocate in parallel fashion to ensure the integrity of the model. For instance, research in animals could identify behavioral phenotypes that distinguish between animals that could be construed as lonely or not, and, in parallel, research in humans could generate, as nearly as possible, an equivalent behavioral phenotype in humans that does not rely solely on explicit self-reports of isolation or loneliness. If the human behavioral phenotype of loneliness exhibits a similar pattern of correlations with outcomes as is exhibited with self-reported loneliness, this may permit greater comparability of findings across animal and human phenotypes.

Finally, the matchless advantage of an animal model of human isolation is the capacity to conduct invasive experimental studies that

provide inroads into mechanisms for the effects of isolation on HPA activity and its diverse consequences in humans AND animals. One of the mechanistic questions of interest is the extent to which social isolation has a direct effect on pro- and anti-inflammatory gene expression, including expression of genes in the glucocorticoid receptor family, and an indirect effect on gene expression through the effects of social isolation on circulating glucocorticoids and other neuroendocrine and immune signals. Beyond gene expression, a mechanistic question of interest is how, when, and in which tissue(s) altered gene expression is translated into changes in functional proteins.

HPA activity has the potential to exert wide-ranging short-term and long-term effects on much of vertebrate physiology. The potential health implications for humans are substantial. We challenge researchers to undertake both animal and human studies with heightened attention to the development of a unified model of social isolation, the integration of findings across species, and the specification of boundary conditions that account for differences among species.

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